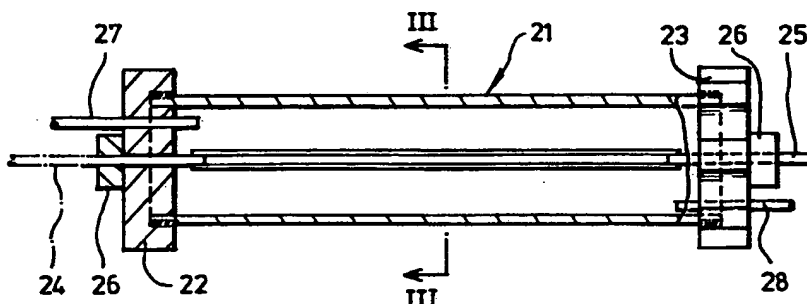




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(54) Title: PREPARING GRAFTS FOR IMPLANTATION**(57) Abstract**

Method and apparatus for preparing a synthetic graft, e.g. a vascular graft, for implantation by lining it with endothelial cells, in which a cell suspension in culture medium is in contact with the graft and the cells incubated whilst maintaining a small shear stress at the medium/graft boundary whereby to cause the cells to become confluent and flatten thus enhancing their attachment to the graft surface. The graft can be placed in a cylindrical chamber rotated to effect the shear force.

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PREPARING GRAFTS FOR IMPLANTATION

This invention relates to methods and apparatus for preparing grafts, for example synthetic or biological vascular grafts, for implantation into human or animal patients.

Synthetic vascular grafts, which may be made for example of polytetrafluoroethylene (PTFE) are implanted when the patient's own tissue is not available though animal, e.g. bovine, grafts can also be used. For non-thrombogenic grafts it is desirable that the PTFE be covered by the patient's own or other non-rejectable endothelial cells. While endothelial cell growth occurs to bare PTFE implants after implantation, this takes place only to a limited extent near the suturing positions.

Endothelial cell seeding has been attempted, but high cell losses occur on implantation once the implant is subjected to blood flow stresses.

The present invention provides methods and apparatus which overcome this problem.

The invention comprises a method for preparing a graft for implantation by lining with endothelial cells,

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comprising placing a cell suspension in a culture medium in contact with the graft and incubating the cells whilst maintaining a small shear stress at the medium/graft boundary whereby to cause the cells to become confluent and flatten thus enhancing their attachment to the graft surface.

The graft may be a vascular graft in the form of a flexible tube. The flexible tube may be held extended substantially horizontally and rotated about its axis to maintain the said shear stress. Or it may be held vertically or in any other orientation, as may any non-tubular graft, in a slow fluid current.

A tubular graft may be coated internally; successive charges of suspension may be introduced into the graft lumen with the graft oriented in different positions about its substantially horizontal axis. The graft may also be coated externally and may be rotated in a cell suspension in a culture medium.

The incubation may be carried on overnight.

The shear stress may be applied by relative movement of the graft and the suspension at a rate of 10^{-5} to 10^{-4} ms^{-1} . This corresponds, with a horizontally extended tube, to a rotation about its axis of the order of 10 rph.

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The invention also comprises apparatus for preparing a graft for implantation by lining with endothelial cells, comprising a chamber in which the graft can be contained and means for flowing a cell suspension in culture medium over a surface of the graft so as to create and maintain a small shear stress at the medium/graft boundary.

Apparatus according to the invention for lining a vascular graft with endothelial cells may comprise a chamber with provision for cannulae on which the ends of the graft are placed and between which the graft is extended whereby cell suspension can be introduced into and removed from the graft lumen. A cylindrical chamber being adapted for rotation about its axis, so as to bring about the necessary small shear effect. However, the chamber can be arranged vertically or in any other orientation and the medium with suspended cells flowed through the graft.

The chamber may be adapted for rotation about its axis by end closures doubling as support means for use on a roller table or by tyres at or intermediate its ends.

End closures may comprise inlet and outlet apertures for a suspension of cells contacting the exterior surface of the graft.

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The apparatus may be supplied with a graft mounted therein ready for preparation for implantation, the whole being already sterilized, if desired, for example by gamma irradiation.

Methods and apparatus for preparing synthetic grafts for implantation according to the invention will now be described with reference to the accompanying drawings, in which :-

Figure 1 is a lengthwise section of a vascular graft showing internal and external cell coatings;

Figure 2 is a lengthwise section of a chamber for preparing a vascular graft, with a graft in place;

and Figure 3 is a cross-section on the line III-III of Figure 2.

The drawings illustrate methods and apparatus for preparing a synthetic vascular graft 11 for implantation by lining with endothelial cells 12, Figure 1.

The endothelial cells may be harvested from the patient, where this is possible, or from umbilical veins

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otherwise. An umbilical vein is cannulated at either end and flushed with 10 ml minimum essential medium (MEM) to remove old blood. The vein is then distended with 5 ml 0.1% collagenase solution prewarmed to 37°C and the whole cord incubated for 15 min at 37°C. After incubation the vein is flushed through with 20 ml MEM to remove dislodged endothelial cells and the resulting cell suspension centrifuged for 7 min at 200 g and 4°C. The resulting cell pellet is resuspended in 5 ml complete medium with 20 ug/ml endothelial growth supplement and 90 ug/ml heparin and the cells placed on to a 25 cm² tissue culture flask and incubated at 37°C in 95% air/5% CO₂ for 3-7 days until they reach confluence. Once at confluence they are harvested with 0.1% trypsin/0.02% ethylene diaminetetracetic acid at 37°C. The trypsin is then inhibited by adding excess MEM containing 20% fetal calf serum. The resulting cell suspension is centrifuged for 7 min at 200 g and 4°C and the cell pellet resuspended in 8 ml complete medium at a concentration of 1.25×10^5 cells/ml.

Figures 2 and 3 illustrate a cylindrical treatment chamber 21 having removable end closures 22,23 with apertures for cannulae 24,25 of which cannula 24 is somewhat longer than cannula 25. One end of the graft 11 is placed on cannula 24 which is pushed through the closure 22 so that some length of it projects into the

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chamber 21 when the closure 22 is fitted to it. The length of the chamber is suited to the length of the graft (the longest graft normally required for human patients being one metre, other grafts being of different shorter lengths so that a range of cylinder lengths will be required) so that the other end of the graft 11 projects some distance beyond the other end of the chamber 21 so as to facilitate its cannulation by cannula 25. The closure 23 is then fitted to the cylinder 21 and cannula 24 drawn back out of the cylinder 21 to tighten the graft 11 and extend it between the two cannulae.

The cannulae 24,25 are sealed and secured in position in the end closures 22,23 by screw clamps 26.

Inlet and outlet apertures 27,28 are provided in the end closures 22,23 for introducing a support medium so that the graft is at neutral buoyancy during the procedure - the support medium can be simply a cell culture medium that can infuse into the graft and assist in the growth of cells on the inner wall or may include suspension of endothelial cells to coat the exterior surface of the graft 11. Obviously where this optional measure is adopted, rather more than the 8 ml aforementioned of cell suspension will be required.

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For coating the interior of the graft, assuming a 4 mm internal diameter graft 15 cm long, 2 ml of the suspension is introduced into the graft lumen from a syringe via the end cannula. The chamber 21 is laid horizontally in an incubator at 37°C for 20 min, after which the medium containing any unattached endothelial cells is removed. The chamber is then rotated 90° about its axis and a further 2 ml of cell suspension introduced as before, and the chamber incubated a further 20 min. The procedure is repeated twice more until all 8 ml of suspension has been used, the final aliquot being left in the graft lumen and the chamber placed on a roller table and rotated at 10 rph during overnight incubation.

Before implantation, the final aliquot is removed from the graft lumen.

Clearly, if desired, the exterior surface of the graft can be coated in the same way at the same time.

The end caps 24,25 are profiled to act as supports for the chamber on the roller table (not shown).

Whilst the chamber 21 may be made of durable, autoclavable materials for repeated use, it is possible to realise them as disposable items pre-loaded with

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graft so that the graft-mounting operation may be effected under factory conditions and with factory quality assurance rather than using hospital resources. A pre-pack may be sterilized by gamma irradiation before or after packaging.

Of course, the coating procedure will be carried out in the hospital because it is not desirable that the coated graft is left for long before implantation.

The 10 rph rotation during incubation applies a small shear stress to the endothelial cell layer and this, together with the incubation, gives a flattened, confluent lining which resists detachment of cells under blood flow conditions. Some cell detachment is observed in trials, but better than 80% of cells are retained.

Where time is short, the overnight incubation and rotation can be omitted. The procedure to that stage is effective at coating the graft, but substantially higher rates of detachment are experienced.

The rotational speed may be different from the 10 rph quoted - speeds as high as 2 rpm have been used effectively.

Various modifications can be made to the means for carrying out the lining according to the invention.

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Instead of relying on a rotation-induced shear stress, suspension may be flowed over or through the graft by pumping or by convection, and this will in general be more useful for non-tubular grafts.

CLAIMS

1. A method for preparing a graft for implantation by lining with endothelial cells, comprising placing a cell suspension in a culture medium in contact with the graft and incubating the cells whilst maintaining a small shear stress at the medium/graft boundary whereby to cause the cells to become confluent and flatten thus enhancing their attachment to the graft surface.

2. A method according to claim 1, in which the graft is a vascular graft in the form of a flexible tube.

3. A method according to claim 2, in which the flexible tube is held extended substantially horizontally and rotated about its axis to maintain the said shear stress.

4. A method according to claim 2 or claim 3, in which the graft is coated internally.

5. A method according to claim 4, in which successive charges of suspension are introduced into the graft lumen with the graft oriented in different positions about its substantially horizontal axis.

6. A method according to any one of claims 2 to 5, in which the graft is coated externally.

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7. A method according to claim 6, in which the graft is rotated in a cell suspension in a culture medium.

8. A method according to any one of claims 1 to 7, in which the incubation is carried on overnight.

9. A method according to any one of claims 1 to 8, in which the shear stress is applied by relative movement of the graft and the suspension at a rate of 10^{-5} - 10^{-4} /ms⁻¹.

10. A method according to claim 9, in which the graft is a horizontally extended tube maintained in rotation at about 10 rph.

11. Apparatus for preparing a graft for implantation by lining with endothelial cells comprising a chamber in which the graft can be contained and means for flowing a cell suspension in culture medium over a surface of the graft so as to create and maintain a small shear stress at the medium/graft boundary.

12. Apparatus according to claim 11 for filing a vascular graft with endothelial cells, comprising a chamber with provision for cannulae on which the ends of the graft are placed and between which the graft is

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extended whereby cell suspension can be introduced into and removed from the graft lumen.

13. Apparatus according to claim 11, in which the chamber is cylindrical and adapted for rotation about its axis so as to bring about the necessary small shear effect.

14. Apparatus according to claim 13, having end closures with apertures for cannulae.

15. Apparatus according to claim 14, the end closures doubling as support means for use on a roller table.

16. Apparatus according to any one of claims 12 to 15, comprising inlet and outlet apertures for a suspension of cells contacting the exterior surface of the graft.

17. Apparatus according to any one of claims 11 to 16, with a graft mounted therein, ready for preparation for implantation.

18. Apparatus according to claim 17, sterilized.

19. Apparatus according to claim 18, gamma-irradiated.

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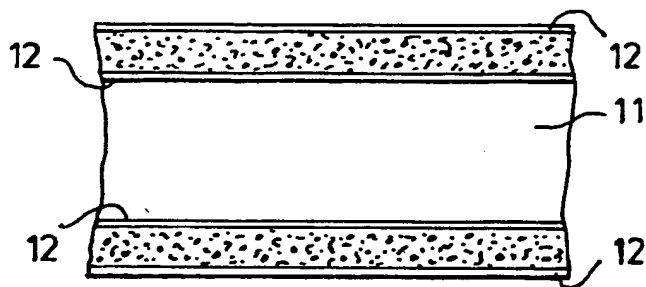


FIG. 1

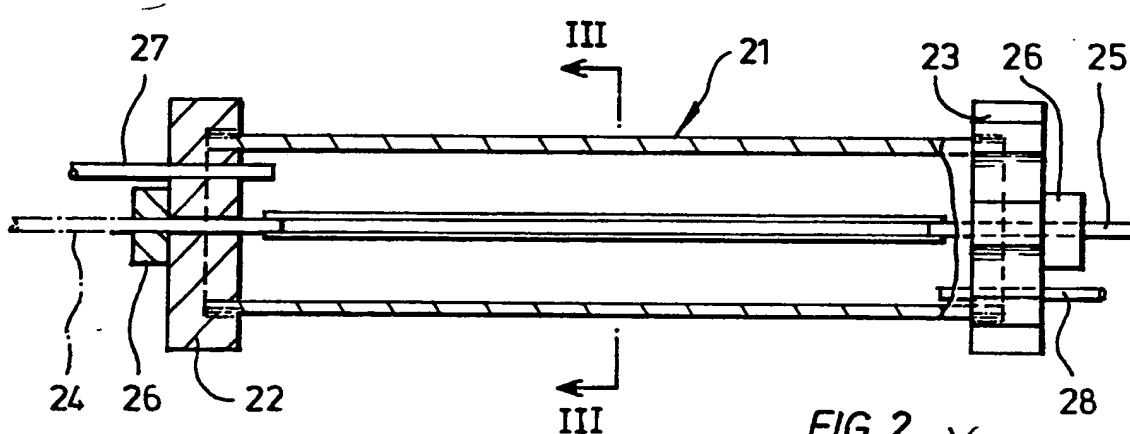


FIG. 2 X

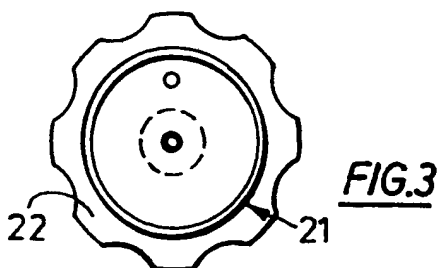


FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/01364

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: A 61 L 33/00														
II. FIELDS SEARCHED <div style="text-align: center;">Minimum Documentation Searched⁷</div> <table style="width: 100%; border: none;"> <tr> <td style="width: 25%; border: 1px solid black; padding: 5px;">Classification System</td> <td style="border: 1px solid black; padding: 5px;">Classification Symbols</td> </tr> <tr> <td style="border: 1px solid black; padding: 5px;">IPC5</td> <td style="border: 1px solid black; padding: 5px;">A 61 L</td> </tr> </table> <div style="text-align: center; padding: 5px;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched⁸</div>			Classification System	Classification Symbols	IPC5	A 61 L								
Classification System	Classification Symbols													
IPC5	A 61 L													
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; padding: 5px;">Category *</th> <th style="width: 60%; padding: 5px;">Citation of Document,¹¹ with indication, where appropriate, of the relevant passages¹²</th> <th style="width: 30%; padding: 5px;">Relevant to Claim No.¹³</th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">EP, A1, 0348969 (BECTON DICKINSON AND COMPANY) 3 January 1990, see page 8, line 31 - line 44; claim 4 ---</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-19</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">WO, A1, 8203764 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 11 November 1982, see page 9, line 7 - page 11, line 27; figure 2; claim 4 ---</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-19</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;">WO, A1, 9116009 (CURATIVE TECHNOLOGIES, INC.) 31 October 1991, see the whole document -----</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-19</td> </tr> </tbody> </table>			Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	Y	EP, A1, 0348969 (BECTON DICKINSON AND COMPANY) 3 January 1990, see page 8, line 31 - line 44; claim 4 ---	1-19	Y	WO, A1, 8203764 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 11 November 1982, see page 9, line 7 - page 11, line 27; figure 2; claim 4 ---	1-19	A	WO, A1, 9116009 (CURATIVE TECHNOLOGIES, INC.) 31 October 1991, see the whole document -----	1-19
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A	WO, A1, 9116009 (CURATIVE TECHNOLOGIES, INC.) 31 October 1991, see the whole document -----	1-19												
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>* Special categories of cited documents:¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>														
IV. CERTIFICATION <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: 1px solid black; padding: 5px;"> Date of the Actual Completion of the International Search 4th November 1992 </td> <td style="width: 50%; border: 1px solid black; padding: 5px;"> Date of Mailing of this International Search Report <div style="text-align: center; font-size: 1.2em;">13 NOV 1992</div> </td> </tr> <tr> <td style="border: 1px solid black; padding: 5px;"> International Searching Authority <div style="text-align: center;">EUROPEAN PATENT OFFICE</div> </td> <td style="border: 1px solid black; padding: 5px;"> Signature of Authorized Officer <div style="text-align: center;">Sofia Nikolopoulou</div> </td> </tr> </table>			Date of the Actual Completion of the International Search 4th November 1992	Date of Mailing of this International Search Report <div style="text-align: center; font-size: 1.2em;">13 NOV 1992</div>	International Searching Authority <div style="text-align: center;">EUROPEAN PATENT OFFICE</div>	Signature of Authorized Officer <div style="text-align: center;">Sofia Nikolopoulou</div>								
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**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 92/01364**

SA 62911

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A1- 0348969	03/01/90	AU-B- 620621	20/02/92
		AU-D- 3598989	04/01/90
		JP-A- 2065867	06/03/90
		US-A- 4927676	22/05/90
WO-A1- 8203764	11/11/82	EP-A-B- 0078314	11/05/83
		US-A- 4539716	10/09/85
		US-A- 4546500	15/10/85
WO-A1- 9116009	31/10/91	AU-D- 7792591	11/11/91

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